Fate of organic carbon released from decomposing copepod fecal pellets in relation to bacterial production and ectoenzymatic activity

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ABSTRACT: Fecal pellets were produced by *Acartia tonsa* fed ¹⁴C-labeled diatom, cryptophyte, and dinoflagellate diets, and were incubated in 1.2 µm-filtered Long Island Sound seawater. Based on the ¹⁴C label, the decrease in fpOC (fecal pellet organic carbon), the release and fate of dissolved organic carbon (DOC) and particulate organic carbon (POC), as well as bacterial production and enzymatic activity, were followed over a 96 h period. fpOC decreased by 9, 14, and 19 % d⁻¹ in diatom, cryptophyte, and dinoflagellate pellets, respectively. There was a fast, possibly passive, leakage of DOC from pellets from all 3 diets within a few hours after egestion, which may not have been utilized by attached bacteria. Bacterial production rates were 17, 12, and 31 pg C pellet⁻¹ h⁻¹, on diatom, cryptophyte, and dinoflagellate pellets, respectively. These were 5 orders of magnitude higher than production rates of free-living bacteria, indicating that copepod fecal pellets are hot spots of pelagic microbial production. The high production was caused primarily by high initial bacterial abundances. Accordingly, production and growth were entirely uncoupled in diatom pellets. There were no increases in abundance of attached bacteria on any of the 3 diets, indicating that the produced bacterial cells were released from the fecal pellets. Attached bacteria had a higher ectoenzymatic activity than free-living bacteria, but their production and ectoenzymatic activity were uncoupled and they only assimilated a minor fraction of the released DOC. DOC was therefore released favoring free-living microbes. The chitinase activity, which increased several-fold, was coupled to the production of attached bacteria; thus, chitin may play an important role in bacterial production on copepod fecal pellets.

KEY WORDS: Copepod fecal pellets · Fecal pellet decomposition · Pelagic DOC flux · Pelagic POC flux · Attached bacterial production · Ectoenzymatic activity

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INTRODUCTION

Fecal pellets originating from mesozooplankton constitute an important pool of particulate organic matter in the epipelagic ocean. Although fecal pellet in some cases constitute a significant fraction of the sedimenting organic carbon (Gonzalez et al. 1994, Landry et al. 1994), they typically contribute less to the sedimentary flux than other sources (Fowler et al. 1991, Wassmann et al. 1999, Roy et al. 2000). Accordingly, many studies report low contributions from fecal pellets to the lower

ocean proper (Bathmann et al. 1987, Lane et al. 1994, Lundsgaard & Olesen 1997) even with high secondary production in the euphotic zone (Ayukai & Hattori 1992, Viitasalo et al. 1999). However, the significance of zooplankton fecal pellets extends well beyond the question of carbon sequestration. The disparity between fecal pellet production and contribution to the sedimentary flux is partly a function of the recycling of organic matter originating from fecal pellets. It is now generally believed that organic matter originating from fecal pellet produced by small copepods is re-

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cycled (Wotton & Malmqvist 2001, Turner 2002) and that most carbon originating from these fecal pellets remains part of the long-lived organic carbon in the epipelagic (Legendre & Michaud 1998). Hence, the role of fecal pellets from small copepods is that of supplying nutrients to the epipelagic planktonic microbial community rather than maintaining the vertical flux of organic matter.

Copepod fecal pellets host an extensive flora of attached bacteria (Gowing & Silver 1983, Bianchi et al. 1992, Delille & Razouls 1994, Hansen & Bech 1996). Breakdown of the fecal pellets is in part governed by microbial decomposition driven by the hydrolytic activity of these bacteria. The potential hydrolytic activity is generally high in pelagic aggregates of organic matter (Karner & Herndl 1992, Smith et al. 1992), and if this potential is fully exploited in fecal pellets, then decomposition and hence recycling of organic matter may occur rapidly. The fate of the hydrolyzed organic compounds can be manifold. For example, the organic matter can be incorporated into attached bacteria, thereby remaining associated with the fecal pellets. Conversely, the rates of hydrolytic activity and production of attached bacteria may be uncoupled (Smith et al. 1992), resulting in the release of DOC. This might nurture the growth of non-attached free living bacteria and protozoans (Cho & Azam 1988).

In the present study we investigated the fate of fecal pellet organic matter in relation to ectoenzymatic activity and growth of attached bacteria. Using the approach of Hansen et al. (1996), we studied the decomposition of fecal pellets produced by *Acartia tonsa* fed on 3 different algal diets that represented the phytoplankton succession through a typical spring–summer season in temperate waters: *Thalassiosira weissflogii* represented a spring diatom bloom situation, the cryptophyte *Rhodomonas lens* the summer phytoplankton dominated by nanoflagellates, and *Prorocentrum minimum* a late-summer dinoflagellate bloom.

MATERIALS AND METHODS

Production and labeling of fecal pellets. Strains of *Thalassiosira weissflogii*, *Rhodomonas lens*, and *Prorocentrum minimum* were kept in the exponential growth phase in f/2 medium (Guillard & Ryther 1962) on an 18:6 h light:dark cycle at 16°C and 34‰ S. Silica was added to *T. weissflogii*, and *R. lens* was bubbled gently to maintain exponential growth. Prior to the experiments, the algae were diluted with f/2 medium and 600 $\mu\text{Ci Na}^{14}\text{CO}_3 \text{ l}^{-1}$. They were then grown for at least 4 generations to ensure uniform isotope labeling (Nielsen & Olsen 1989).

Specimens of the copepod *Acartia tonsa* were caught in Long Island Sound and kept in culture on a mixture of the 3 algal species in dim daylight conditions at 20°C and 34‰ S. Adult copepods and late copepodites from this culture were acclimated with the algal species used in the experiments for 24 h. Approximately 500 acclimated copepods were then retrieved on a 200 μm monofilament screen and placed in two 5 l beakers holding the labeled algae (500 $\mu\text{g C l}^{-1}$). Screen insets (200 μm mesh-size) at the bottom allowed fecal pellets to escape coprophagy. Gentle bubbling kept the algae in suspension. The copepods were then allowed to feed on the labeled algae for 6 h, a sufficiently long period to ensure complete evacuation of unlabeled material from the gut (Dam & Peterson 1988). Unlabeled fecal pellets were removed and the copepods were re-introduced to the labeled algae at the same concentration. After 3 to 5 h the labeled fecal pellets were retrieved on a 30 μm screen. The fecal pellets were then washed with 0.5 μm filtered seawater (fsw) twice to remove any dissolved label and transferred to fsw in sterilized petri dishes.

Water for the experiments was collected at 4 m depth in Long Island Sound and filtered through 1.2 μm glass-fiber filters (filtered LIS water), and 100 labeled and intact fecal pellets were mouth-pipetted from the petri dishes into sterilized vials holding 10 ml of this water. Pasteur pipettes drawn out over a flame to obtain an opening not much larger than the fecal pellets themselves were used for the pipetting. The accuracy of the pipetting procedure was 5 %. This was tested by counting the pipetted fecal pellets. We incubated 3 replicates for carbon release measurements and 3 replicates for bacterial production measurements for 0, 12, 24, 48, and 96 h, respectively. All vials were placed on a tilting table rotating gently at 30 rpm to mimic water movement around sinking fecal pellets. The water movement was gentle enough to avoid mechanical breaking of the fecal pellets but sufficient to ensure total mixing of the water in the vials within a few minutes; this was tested with dye. To determine the direct contribution of dissolved ^{14}C -label from the copepod culture, 3 replicate controls of the same volume but without fecal pellets were pipetted directly into scintillation vials and measured by liquid scintillation.

Carbon release. After the incubation, all samples were preserved by pipetting 600 μl of 34 % formaldehyde into the vials to a final concentration of 2 %. The samples were then split into 2 ml aliquots containing fecal material (fecal pellet aliquots) and 5 ml aliquots containing no visually discernible fecal material (non-fecal pellet aliquots). This was done by pipetting 8 ml from the vials holding the preserved samples into a petri dish without disturbing the fecal pellets at the bottom of the vials, examination at 40× magnification for fecal remnants, and

pipetting a 5 ml aliquot of this 8 ml sample into other vials. All aliquots were filtered through 0.45 µm cellulose acetate filters at low suction to allow measurement of $PO^{14}C$ (filter) and $DO^{14}C$ (filtrate) by liquid scintillation counting after dissolving filters in ethyl acetate. The fecal pellets were thus retrieved on 0.45 µm filters, which should have avoided damage. The organic carbon content of these 4 fractions ($ng\ C\ ml^{-1}$) was calculated by dividing the isotopic activity of the samples (disintegrations $min^{-1}\ ml^{-1}$; $dpm\ ml^{-1}$) with the specific activity of the algae ($dpm\ ng\ C^{-1}$). The specific activity of the algae was calculated by dividing the specific activity of a 1 ml sample of labeled algae of a known concentration filtered onto GF/F filters ($dpm\ cell^{-1}$) by values of carbon content ($pg\ C\ cell^{-1}$). Carbon contents were either obtained from previous CHN analysis or from measurements of equivalent spherical diameter and carbon to volume ratio (Strathmann 1967, Besiktepe & Dam 2002). Thus, 4 different fractions of organic carbon were obtained in each sample: DOC_p ($ng\ DOC\ ml^{-1}$ in fecal pellet aliquots), POC_p ($ng\ POC\ ml^{-1}$ in fecal pellet aliquots), DOC_w ($ng\ DOC\ ml^{-1}$ in non-fecal pellet aliquots), POC_w ($ng\ POC\ ml^{-1}$ in non-fecal pellet aliquots).

These fractions were used to calculate the release and fate of the original fecal pellet organic carbon (in $ng\ C\ pellet^{-1}$). The 3 different carbon pools were calculated as:

$$\begin{aligned} fpOC &= (POC_p - control) \frac{V}{n} \\ DOC &= (DOC_p) \frac{V}{n}, \text{ and} \\ POC &= (POC_w - control) \frac{V}{n} \end{aligned}$$

where $fpOC$ is organic carbon still retained in the fecal pellet, DOC is organic carbon originating from fecal pellets dissolved and released to the surrounding water, and POC is particulate organic carbon originating from fecal pellets released to the surrounding water. V is the incubation volume (10 ml), and n is the number of fecal pellets (100). It follows that since these calculations are based solely on ^{14}C originating from the algae on which the copepods had previously fed, these carbon pools represent only carbon originating from the fecal pellets. All contributions of organic carbon from sources other than the fecal pellets themselves are thereby excluded.

Bacterial production. Bacterial production of the fecal pellet and water fractions was measured using the 3H -thymidine incorporation technique (Bell 1993). One hour before the end of the incubations, 200 µl of 20 µM methyl- 3H -thymidine ($50\ Ci\ mmol^{-1}$) was added to the replicates. At the end of the incubations, the samples were fixed with 600 µl of 34 % formaldehyde and 10 fecal pellets were removed for enumeration of attached

bacteria. An aliquot of 4 ml including fecal pellets was taken for production measurement of attached bacteria (bp_p). Another aliquot of 4 ml not including any fecal material (ascertained at 40× under the dissecting microscope) was taken for production measurement of free-living bacteria (bp_w). We added 400 µl of 50 % trichloroacetic acid (TCA) to all samples, which were kept on ice for 15 to 60 min, and subsequently filtered through 1 mM thymidine-soaked 0.45 µm cellulose acetate filters. Following rinsing with 1 ml ice-cold 5 % TCA (×3) and 1 ml ice-cold 80 % ethanol (×5), the filters were dissolved in ethyl acetate and counted for isotopic activity using a truncated counting window to minimize interference from ^{14}C . Blanks taken at the beginning of the incubation period were treated similarly. Bacterial production rates were calculated according to Bell (1993) using a thymidine conversion factor (TCF) of 2×10^{18} cells mol^{-1} . To exclude the contribution from free-living bacteria to the production of attached bacteria, bp_w was subtracted from bp_p . Growth rates of attached bacteria were calculated by dividing bacterial production rates by bacterial abundances. Unfortunately we did not measure the abundance of free-living bacteria. The samples for bacterial enumeration were sonicated in 5 ml 5 mM Na_4PPI to release the bacteria from the fecal pellets (Velji & Albright 1993) and counted under an epifluorescence microscope at 1250× magnification using the acridine orange direct count (AODC) method (Hobbie et al. 1977).

Ectoenzymatic activity. Extracellular enzymatic activity was measured using several different substrates attached to fluorescent markers, either 4-methylumbelliferyl (MUF) or 4-methylcoumarinyl-7-amide (MCA) (Table 1). When they are released from the substrate, the markers become fluorescent by enzymatic cleavage (Hoppe 1993). Fecal pellets from the two 5 l beakers (see subsection 'Production and labeling of fecal pellets' above) were pipetted into plastic cuvettes holding 2 ml-filtered LIS water, with 20 fecal pellets in each. The experiments were conducted with 4 replicate fecal pellet samples and 4 replicate filtered LIS water samples of 5 different substrates at a concentration of

Table 1. Cursor-substrate complexes used in ectoenzymatic activity assays. MUF: 4-methylumbelliferyl; MCA: 4-methylcoumarinyl-7-amide

Cursor-substrate complex	Substrate	Enzyme
MUF- α -D-glucopyranoside	Glucogen	β -glucosidase
MUF- β -D-glucopyranoside	Cellulose	β -glucosidase
MUF-N-acetyl-glucosaminide	Chitin	Glucosaminidase
MUF-phosphate	Lipid	Phosphatase
MCA-L-leucine	Protein	Leucine aminopeptidase

75 μM . The cuvettes were incubated for a total of 96 h. At 0, 12, 24, 48, and 96 h the fluorescence of the samples was read in a Hitachi F-2500 fluorescence spectrophotometer. The fluorescence was measured at 364 nm excitation and 445 nm emission for MUF and 380 and 440 nm for MCA. The rate of hydrolysis (nM h^{-1}) was calculated according to Hoppe (1993) using standard curves obtained with known concentrations of MUF and MCA standards.

All statistical tests were performed using either StatView 5.01 (SAS Institute) or SigmaStat 2.03 (SPSS).

RESULTS

The initial masses of the fecal pellets (sum of the fpOC, POC, and DOC pools) were $12.8 \pm 2.1 \text{ ng C pellet}^{-1}$ (mean $\pm$ SE) on the diatom *Thalassiosira weissflogii* diet, $58.3 \pm 19.7 \text{ ng C pellet}^{-1}$ on the cryptophyte *Rhodomonas lens* diet, and $68.4 \pm 7.4 \text{ ng C pellet}^{-1}$ on the dinoflagellate *Prorocentrum minimum* diet. The total amount of organic carbon originating from the pellets (fpOC + DOC + POC) varied during the incubation period. Logically, this should not change by more than what is lost by respiration, since we measured all pools of organic carbon originating from the pellets. The variation was quite high in pellets from the diatom diet (66% between the highest and lowest value), but

lower in pellets from cryptophyte (15%) and dinoflagellate (29%) diets. There was, nevertheless, no significant difference between the total carbon mass at the different sampling points during the incubations on all 3 diets (1-factor ANOVA, $p > 0.05$) and the mean ($\pm$ SE) total carbon masses of the whole incubation periods of 96 h were almost identical to the initial masses (diatom: $11.7 \pm 1.1 \text{ ng C pellet}^{-1}$, cryptophyte: $52.1 \pm 1.0 \text{ ng C pellet}^{-1}$, dinoflagellate: $74.4 \pm 2.8 \text{ ng C pellet}^{-1}$).

Decomposition of fecal pellets

During the 96 h incubation period, there was no significant decrease in fpOC in pellets from the diatom diet (hereafter called 'diatom pellets') (1-factor ANOVA, $p > 0.05$; Fig. 1, Table 2). Conversely, decomposition was more pronounced in pellets from the cryptophyte and dinoflagellate diets (hereafter called 'cryptophyte' and 'dinoflagellate pellets'). In cryptophyte pellets, fpOC decreased significantly from 36.9 to 23.1 ng C pellet^{-1} (1-factor ANOVA: $F = 5.24$, $df = 4$, $p = 0.018$), and in dinoflagellate pellets it decreased significantly from an initial 38.6 to 19.1 ng C pellet^{-1} at 96 h (1-factor ANOVA: $F = 7.36$, $df = 4$, $p = 0.005$). The fpOC decrease rates were significantly different between cryptophyte and dinoflagellate pellets (comparison of slopes: Student's $t = 2.74$, $df = 27$, $p < 0.02$).

Fig. 1. Carbon content of the 3 pools, fpOC (fecal pellet organic carbon), DOC and POC during the 96 h incubation period. Fecal pellets produced by *Acartia tonsa* on diets of (a) diatom *Thalassiosira weissflogii*, (b) cryptophyte *Rhodomonas lens*, (c) dinoflagellate *Prorocentrum minimum*. Error bars = SD; $n = 3$

Fate of released carbon

The amounts of released DOC were already high at the onset of the incubation period. This was true for all 3 diets, indicating that 50, 34, and 42 % of the total carbon content (fpOC + DOC + POC) had leaked from diatom, cryptophyte, and dinoflagellate pellets at Time $t = 0$, i.e. during the initial handling of the pellets. In diatom and dinoflagellate pellets this release continued over the next 12 h. After this initial release, DOC levels decreased on all 3 diets followed by another increase after 48 h (Fig. 1). The timing of these events was similar for diatom and dinoflagellate pellets, with peak DOC levels after 12 h. For cryptophyte pellets, a peak occurred earlier, at the beginning of the incubation period. However, none of these changes in DOC levels were significant (1-factor ANOVAs: $p > 0.15$).

The amounts of organic carbon originating from the fecal pellets that were recovered from the POC fractions (POC not including fecal pellets) were low compared to the DOC fractions. Thus, on average over the incubation period, only 6 % of the total organic carbon was recovered as non-pellet POC in diatom pellets, 6 % in cryptophyte pellets, and 1 % in dinoflagellate pellets. Nevertheless, of the total amount of organic carbon released from the pellets (DOC+POC) 25 % of the diatom pellet carbon and 21 % of the cryptophyte pellet carbon were incorporated into non-pellet POC. In cryptophyte pellets the 6.4 ngPOC pellet⁻¹ peak at 48 h was significantly different from the initial level (Kruskal-Wallis 1-factor ANOVA by ranks: $H = 9.956$, $df = 4$, $p = 0.041$).

Bacterial production

Growth rates of attached bacteria ranged from 0.016 to 0.116 h⁻¹, and were highest in diatom and dinoflagellate pellets (Fig. 2). The rates peaked between 24 and 48 h on all 3 diets, averaging 0.102 h⁻¹ in diatom pellets, 0.047 h⁻¹ in cryptophyte pellets, and 0.116 h⁻¹ in dinoflagellate pellets. Additionally, we found higher production rates at 12 h in diatom pellets.

Table 2. Carbon decomposition rate (mean $\pm$ SE; $n = 15$) of fecal pellets produced by *Acartia tonsa* on the 3 diets. Decomposition rate (R) defined as slope of exponential regression $C = C_0 e^{-Rt}$ (Hansen et al. 1996). L-ratio = decomposition rate R (h⁻¹) divided by sinking rate (from Feinberg & Dam 1998) in m h⁻¹

Diet	Carbon decomposition R (h ⁻¹)	r^2	p	Half life t (h)	L-ratio (m ⁻¹)
<i>Thalassiosira weissflogii</i>	0.0038 $\pm$ 0.0048	0.06	0.579	182	2.9 $\times 10^{-3}$
<i>Rhodomonas lens</i>	0.0059 $\pm$ 0.0015	0.59	0.010	117	7.1 $\times 10^{-3}$
<i>Prorocentrum minimum</i>	0.0081 $\pm$ 0.0014	0.77	<0.001	85	9.7 $\times 10^{-3}$

Fig. 2. Production and growth rates of attached bacteria during the 96 h incubation period on fecal pellets produced by *Acartia tonsa* on diets of (a) diatom *Thalassiosira weissflogii*, (b) cryptophyte *Rhodomonas lens*, (c) dinoflagellate *Prorocentrum minimum*. Error bars = SD; $n = 3$

The maximal bacterial production rates were 30.6 pg C pellet⁻¹ h⁻¹ for attached bacteria (Fig. 2) and 867 pg C ml⁻¹ h⁻¹ for free-living bacteria (Fig. 3). Average production rates of attached bacteria were 5 orders of magnitude higher than production rates of free-living bacteria in a similar volume of water. In other words, on average, the bacterial production on a single copepod fecal pellet equalled the production of free-living bacteria occupying a volume of water of 35 μ l.

Production rates of attached bacteria were initially high in diatom pellets (17.1 pg C pellet⁻¹ h⁻¹), but decreased within the first 24 h to a low rate of 6.2 pg C pellet⁻¹ h⁻¹ (Fig. 2). The peak in bacterial growth rate at 24 h did not result in higher production rates. In cryptophyte pellets, the maximal growth rates from 24 to 48 h induced maximal production rates averaging 12 pg C pellet⁻¹ h⁻¹. Production rates of attached bacteria in dinoflagellate pellets peaked similarly at 30.6 pg C pellet⁻¹ h⁻¹ at 48 h. Production rates of attached bacteria were significantly correlated to bacterial growth in cryptophyte and dinoflagel-

Fig. 3. Production of free-living bacteria during the 96 h incubation period on fecal pellets produced by *Acartia tonsa* on diets of (a) diatom *Thalassiosira weissflogii*, (b) cryptophyte *Rhodomonas lens*, (c) dinoflagellate *Prorocentrum minimum*. Error bars = SD; n = 3

late pellets (Pearson's product-moment correlation: cryptophyte: $r = 0.949$, $df = 5$, $p = 0.014$, dinoflagellate: $r = 0.94$, $df = 5$, $p = 0.017$), but not in diatom pellets ($r = -0.20$, $df = 4$, $p = 0.805$). There were no correlations between bacterial production and DOC release on any of the 3 diets (Pearson's product-moment correlation: $p > 0.4$). Production of free-living bacteria followed the amount of POC originating from diatom and cryptophyte pellets. There was a significant correlation in the cryptophyte pellets but not in the diatom pellets (Spearman's rank-order correlation: cryptophyte: $r = 0.514$, $df = 15$, $p = 0.048$, diatom: $r = 0.518$, $df = 11$, $p = 0.095$).

The abundance of attached bacteria ranged from 1.5×10^4 to 4.8×10^4 cells pellet⁻¹ (Fig. 4). Bacterial production did not induce increases in abundances on any of the diets. In fact, bacterial abundance decreased significantly from 24 to 48 h in diatom pellets (1-factor ANOVA: $F = 7.24$, $df = 4$, $p = 0.009$, Student-Newmann-Keuls post-hoc test: $p = 0.012$). Bacterial abundances were significantly higher in diatom pellets than in cryptophyte and dinoflagellate pellets (1-factor ANOVA on diets: $F = 5.52$, $df = 2$, $p = 0.008$, Student-Newmann-Keuls post-hoc test: diatom/cryptophyte: $p = 0.043$, diatom/dinoflagellate: $p = 0.006$).

Ectoenzymatic activity

The difference in the rate of ectoenzymatic activity between the fecal pellets and filtered seawater (Δv) varied between virtually zero and 278 nM h^{-1} . The activity of all 5 enzymes in the fecal pellets was significantly different from that of filtered LIS water (Figs. 5 & 6) for all 3 diets (2-factor ANOVA on incubation time between fecal pellets and water, log-transformed data; p-values in Fig. 5). During the incubation period, the activity in the fecal pellets increased in all samples parallel to a similar increase in filtered LIS water, the activity of the LIS water being at some points higher than that of the fecal pellets (negative values of the sum of enzymatic activity [Δv_{sum}] in Fig. 6). Negative values for β -glucosidase were recorded on all 3 diets.

There were no significant correlations between ectoenzymatic activity and release of DOC or bacterial production and growth except between the production of attached bacteria and glucosaminidase activity in dinoflagellate pellets (Pearson's product-moment correlation: $r = 0.998$, $df = 4$, $p = 0.002$).

Fig. 4. Abundance of bacteria attached to fecal pellets produced by *Acartia tonsa* on diets of (a) diatom *Thalassiosira weissflogii*, (b) cryptophyte *Rhodomonas lens*, (c) dinoflagellate *Prorocentrum minimum*. Error bars = SD; n = 3

Fig. 5. Difference between rates of ectoenzymatic activities (Δv) in filtered seawater and fecal pellets produced by *Acartia tonsa* on diets of (a) diatom *Thalassiosira weissflogii*, (b) cryptophyte *Rhodomonas lens*, (c) dinoflagellate *Prorocentrum minimum*. p values denote significant differences between log-transformed data from fecal pellets and filtered Long Island Sound (LIS) water. Error bars = SD; n = 4

DISCUSSION

Copepod fecal pellets decomposed at rates of 9, 14 and 19 % d⁻¹ for diatom, for cryptophyte pellets, and for dinoflagellate pellets, respectively. These rates are somewhat higher than those (3 to 10 % d⁻¹) reported for *Acartia tonsa* by Lee & Fisher (1992). Similarly, fecal pellets produced by zooplankton larger than 200 μ m and fed a natural assemblage of seston decomposed at a rate of 5 % d⁻¹ (recalculated from Roy & Poulet 1990). On the other hand, the decomposition rates in the present study were slightly lower than those found previously for *A. tonsa* fed comparable diets (Hansen et al. 1996) and for the larger *Calanus hyperboreus* fed diatoms (Urban-Rich 1999). The initial fecal pellet carbon masses were comparable to those previously reported for fecal pellets of similar size to the ones from our study (Urban-Rich et al. 1998).

The copepod diet had a significant effect on pellet decomposition rates. Diatom pellets decomposed at a significantly lower rate than cryptophyte and dinoflagellate pellets. This contrasts to some extent with the findings of Lee & Fisher (1992), who found comparably low decomposition rates for fecal pellets of *Acartia tonsa* fed another diatom, *Thalassiosira pseudonana*, but who did not find any differences in decomposition rates between diatom and haptophyte pellets. Diatom diets tend to produce dense, rapidly-sinking fecal pellets (Feinberg & Dam 1998), and in the present study the L-ratio, i.e. the ratio between breakdown rate and sinking speed, was markedly lower for diatom pellets

than for cryptophyte or dinoflagellate pellets (Table 2). If our low decomposition rates for diatom pellets are valid, this suggests that copepod grazing and fecal pellet production are potentially more important in the sedimentary carbon flux during diatom blooms. However, coprophagy and coprophagy by flagellates, ciliates, or copepods may alter degradation rates completely (Gonzalez & Smetacek 1994, Turner 2002). The low decomposition rate of diatom pellets in our study could have resulted from leakage of organic carbon during the collection of the pellets prior to incubation, biasing the rate estimates negatively. The initial carbon masses of the diatoms pellets were remarkably low

Fig. 6. Sum of ectoenzymatic activities (Δv_{sum}) of β -glucosidase, glucosaminidase, phosphatase, and leucine aminopeptidase in fecal pellets produced by *Acartia tonsa* on all 3 diets. (*Thalassiosira weissflogii*, *Rhodomonas lens*, *Prorocentrum minimum*)

compared to cryptophyte and dinoflagellate pellets. Nevertheless, we feel confident with the validity of our results, since the diatom pellets appeared smaller in size than the cryptophyte and dinoflagellate pellets. Moreover, lower decomposition rates of diatom pellets have been reported previously (Hansen et al. 1996).

We observed high levels of DOC at the onset of the incubation period for all 3 diets. DOC release continued over the subsequent 12 h from diatom and dinoflagellate pellets. Leaking of organic carbon from fecal pellets is probably mediated by both physical and biological mechanisms. Initially, soluble compounds trapped inside the pellet matrix may leak passively before the onset of any substantial microbial decomposition. Model calculations have shown that the steep concentration gradient between fecal pellets and the surrounding water would cause the soluble organic matter to passively diffuse out within minutes (Jumars et al. 1989). These calculations have been supported to some extent by recent studies showing substantial DOC leakage from fecal pellets produced by *Calanus glacialis* and *C. finmarchicus* within 1 h of leaving the gut (Møller et al. 2003). Consequently, the amount of fecal pellet DOC that is actually available for attached bacteria is variable. The key factor is the timing of bacterial colonization. If indeed fecal bacteria stem from the copepod gut and the pellets are decomposed from the inside (Gowing & Silver 1983, Nagasawa & Nemoto 1988, Delille & Razouls 1994), then the large DOC release will result in high bacterial production in a matter of hours. This was evident in diatom pellets, for which both growth rates and certainly production rates were initially high. On the other hand, if there are no internal bacteria (Honjo & Roman 1978), utilization of the initial DOC release by attached bacteria would be very low, and colonization and thus production would occur gradually (Turner & Ferrante 1979), a scenario resembling that observed for the cryptophyte and dinoflagellate pellets in the present study. After the initial release of DOC, the levels stagnated, possibly as a result of increased uptake due to the onset of attached bacterial production at this time.

The high initial levels of DOC resulted from leakage during the 2 h handling period prior to the first sampling, but leakage probably also occurred during the 3 to 5 h collection period prior to the experiments. Since this DOC is not included in our data, the amounts of released DOC reported probably represents minimum levels.

Production rates of attached bacteria varied as a function of their growth rates on the cryptophyte and dinoflagellate diets. Thus, production of new bacterial biomass was an active process on these pellets, and during the 96 h incubation period bacterial production on fecal pellets produced with the cryptophyte and dinoflagellate diets increased with a timing comparable to what has been found earlier (Jacobsen & Azam 1984). Con-

versely, the initial high bacterial production rates on the diatom diet were caused primarily by high initial abundance, and bacterial growth rates remained low on this diet. Using a growth efficiency of 50% (Kroer 1993), the bacterial carbon demand was approximately 5 ng C in diatom and cryptophyte pellets and 10 ng C in dinoflagellate pellets during the 96 h incubation period. Thus, the total DOC release was between 3 and 6 times higher than the amount that would be taken up by attached bacteria. The DOC levels were inversely correlated to the production of attached bacteria (although not significantly), so that the peak in bacterial production at 24 to 48 h generated a decline in DOC levels. The DOC levels measured in the surrounding water was a result of a balance between DOC released by enzymatic activity and DOC assimilated for bacterial production. Therefore, the increased DOC concentrations during the latter part of the incubation period were the result of high enzymatic activity and low production rates of attached bacteria.

The release of DOC may have boosted the growth rates of free-living bacteria, as previously reported for particular aggregates (Unanue et al. 1998). We recovered 25% of the organic carbon released from the diatom and cryptophyte pellets in the non-pellet POC fraction, which includes free-living bacteria, and correlations showed that 50% of the variations in the production of free-living bacteria could be explained by variations in incorporation of carbon into the POC fraction. Thus, it appears that decomposition of copepod fecal pellets engenders a large supply of DOC to non-attached microbes and the primary role of attached bacteria in the epipelagic carbon flux is in triggering particle solubilization and the release of DOM, as previously proposed by Cho & Azam (1988).

In support of this, the production and growth of attached bacteria were more or less uncoupled from the overall ectoenzymatic activity in pellets from all 3 diets. Attached bacteria can exhibit high rates of uptake of e.g., glucose and amino acids (Ayo et al. 2001), despite low thymidine incorporation rates (Kirchman 1983). Since they have low-affinity uptake characteristics adapted to high substrate concentrations (Ayo et al. 2001), a concentrated organic carbon environment such as copepod fecal pellets, might attune their metabolism to production and release of ectoenzymes rather than to growth. Thus, despite higher production rates, growth rates of attached bacteria could be lower than those of free-living bacteria (Alldredge et al. 1986). Unfortunately we did not measure the abundance of free-living bacteria and could make no comparison. Since the average production rates of attached bacteria were 5 orders of magnitude higher than the production rates of free-living bacteria in a similar volume of water, it would appear that cope-

pod fecal pellets—at least on the basis of production—are microbial hot spots. Nevertheless, this high production may have been due solely to higher bacterial abundance, and we did not record growth rates for bacteria that were confidently higher than those reported for free-living bacteria by Unanue et al. (1998). It thus appears that the high ectoenzymatic activity associated with the fecal pellets enhanced release of DOC rather than growth of attached bacteria, similar to the situation for amorphous aggregates (Smith et al. 1992). This appears to be a general trait of attached bacteria in the pelagic environment, leading to release of DOM from sinking particles (Cho & Azam 1988, Karner & Herndl 1992, Unanue et al. 1998).

The activity of glucosaminidase, which catalyzes the hydrolysis of chitin, increased manyfold, reaching a maximum at 48 h on all 3 diets and was, in contrast to the activity of all other enzymes tested, fairly well coupled to the production of attached bacteria. A host of different chitin-hydrolyzing bacteria is present in the marine environment (Osawa & Koga 1995, Kirchman & White 1999). The peritrophic membrane surrounding fecal pellets consists of chitin, and its breakdown appears to be facilitated by high hydrolytic activity. Microscopic inspection of decaying fecal pellets has revealed that the peritrophic membrane can rupture within 3 h and be completely broken down within 24 h (Honjo & Roman 1978). In the present study, hydrolysis of chitin ceased after 48 h, possibly due to complete decomposition of the membrane. Bacterial production on pellets produced by the cryptophyte and dinoflagellate diets correlated relatively well with the glucosaminidase activity (significantly so in dinoflagellate pellets). Thus, chitin may play an important role in bacterial production associated with copepod fecal pellets. Certainly, hydrolysis of chitin has been shown to support a substantial part of estuarine bacterial production (Kirchman & White 1999).

Production of attached bacteria did not result in increased bacterial abundances on the fecal pellets. Since bacteria show a range of different strategies for reversible attachment and release from surfaces (Marshall 1996), the uncoupling of bacterial production and abundance could be attributable to newly produced bacterial cells being released from the fecal pellets. We did in fact observe a decrease in bacterial abundance on fecal pellets from the diatom diet. Similar theories on cell export have been put forward by Jacobsen & Azam (1984), whose study showed that >90% of the attached bacteria were released from copepod fecal pellets. Sediment trap studies have revealed a paradoxical relationship between a decrease in POM flux down through the water column and a decrease in attached microbial biomass (Karl et al. 1988), whereas if the decomposition of particles is mediated by microbial

breakdown, one would expect increasing microbial biomass with increasing breakdown of the sinking particles. This paradox could be partly due to a continual removal of bacterial cells, which have been produced on and have taken part in the decomposition of the sinking particles, similar to the situation that we believe took place in the fecal pellets in the present study.

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